Journal of Colloid and Interface Science 370 (2012) 94–101

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Properties of aqueous solutions of hydrophobically modified polyethylene imines in the absence and presence of sodium dodecylsulfate

Ismael C. Bellettini, Leandro G. Nandi, Renato Eising, Josiel B. Domingos, Vanderlei G. Machado *, Edson Minatti *

Departamento de Química, Universidade Federal de Santa Catarina, UFSC, Florianópolis, SC 88040-900, Brazil

ARTICLE INFO

Article history:
Received 7 October 2011
Accepted 16 December 2011
Available online 28 December 2011

Keywords:
Polyelectrolyte
Intrinsic viscosity
Polymer–surfactant interactions
Pyrene fluorescence
Hydrophobic effect
Polyethylene imine

ABSTRACT

Four modified hyperbranched polyethylene imines (PEIs) were synthesized by means of the alkylation of PEI. SAXS, viscosity, surface tension, and pyrene fluorescence emission were then used as techniques to examine the conformation and aggregation of the modified PEIs in aqueous solution, in the absence and presence of sodium dodecylsulfate (SDS). Analysis of the SAXS data showed that the radius of gyration decreases with an increase in the alkyl chain length of the polymer, while the viscosity data indicated a decrease in the intrinsic viscosity under the same conditions. The nonmodified PEI was not surface active, while the hydrophobically modified samples showed pronounced surface activity and the presence of hydrophobic domains. On addition of SDS, the onset of the formation of polymer–surfactant complexes was determined, indicating a decrease in the critical aggregate concentration with an increase in the alkyl chain length of the polymer backbone.

1. Introduction

Polyelectrolytes are polymers that are soluble in water and in other polar solvents that have charged or ionizable groups, exhibiting properties similar to both electrolytes and polymers. The presence of ionic groups on the polymeric chain influences its solubility and also its intra- and intermolecular interactions. The intramolecular repulsion between the ionic groups leads to a more extended conformation of the polymeric chain in comparison with that normally observed in neutral polymers [1].

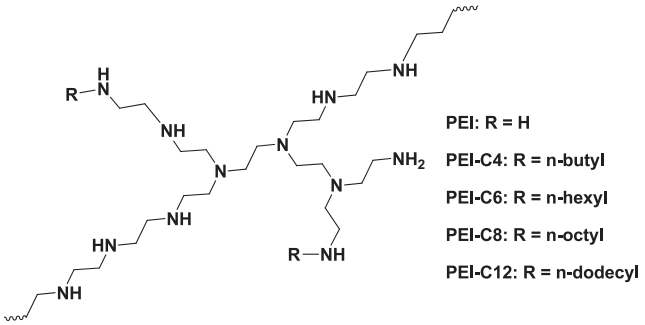
Hyperbranched polyethylene imine (PEI; Scheme 1) is a polyelectrolyte that is used for many purposes, such as in the paper industry [2], in gene delivery therapy [3,4], and in the development of catalyst supports [5–8], being highly charged under low pH conditions and with an increase in pH its charge density decreases. PEI with a molar mass of 25,000 g mol^{-1} is an effective in vitro vector for gene transfer, but its applications in bio-related research are limited due to its cytotoxicity [9]. The latter is mainly associated with the fact that PEI is a strongly charged polycation, which leads to its strong interaction with cell surfaces and consequently to their damage. Therefore, modifications in the polymeric backbone of PEI that attenuate its positive charge might be useful in reducing the toxicity of the polymer [10].

The design of hydrophobically modified polyelectrolytes has attracted research interest in recent years due to their similarity to biological systems, as well as their strong tendency toward self-organization in aqueous solution caused by the combination of electrostatic and hydrophobic effects [1,11]. It is also important to observe that up to 40% of new chemicals discovered by the pharmaceutical industry nowadays are hydrophobic compounds [12]. Since the amino groups of PEI are chemically reactive, they can be readily derivatized by a large number of functional groups, allowing the formation of various versatile decorated polymeric materials adapted to the required application. For instance, the functionalization using long chain alkyl groups affords access to hydrophobically modified PEIs which, in principle, allows the combination of the properties of pure polyelectrolyte polymers with those of the amphiphilic groups appended [13].

The interaction of polyelectrolytes with surfactants of opposite charge in water has been studied by many research groups in recent decades due to the potential technological applications in many fields, such as in the personal care and cosmetic products industry [14], gene therapy [15], drug delivery [16], and water treatment [17–19]. The polyelectrolyte–surfactant association in aqueous media leads to the formation of thermodynamically stable complexes, and the resulting physicochemical features of these entities are different from those observed in pure surfactant micellar medium. Several studies have been carried out on systems comprised of PEI and sodium dodecylsulfate (SDS) [20–28]. They have shown that the pH of the PEI solution increases with increasing SDS concentration, which has been interpreted in terms of favor-

* Corresponding authors. Fax: +55 48 3721 6852.
E-mail addresses: vander@qmc.ufsc.br (V.G. Machado), minatti@qmc.ufsc.br (E. Minatti).

0021-9797 © 2011 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.jcis.2011.12.049



Scheme 1. Structures of PEI and hydrophobically modified PEIs.

able specific interactions involving the protonated amine groups in PEI and the oppositely charged sulfate group in SDS [20–22]. Winnik et al. [21] showed that at low concentrations of SDS, PEI exhibited an exothermic interaction that involved individual SDS molecules and the protonated amine groups, which was responsible for driving an increase in pH. However, at higher concentration of SDS, the interaction is endothermic due to a noncooperative adsorption of SDS micelles onto the polymeric chains. Recently, the formation of supramolecular complexes composed of PEI, sodium cholate, and SDS was investigated by Felippe et al. using various techniques [29].

We have recently exploited the design of covalently modified PEIs aiming at their use as supports for catalysts and also to study their interaction in aqueous solution with DNA. In order to understand more profoundly the role of the PEIs in these complex systems, a study of the behavior of the PEIs in water in the absence and presence of anionic surfactants was addressed. Although the literature reports the use of different techniques to investigate the interaction of PEI with SDS in aqueous solution [20–28], very few papers can be found concerning mixtures of modified PEIs with SDS. Griffiths et al. [30] showed that PEI does not have surface activity, while hydrophobically modified PEI with pendant dodecyl groups exhibits marked surface activity, with the presence of small hydrophobic domains being observed. An increase in the degree of PEI modification led to an increase in the surface activity of the system under study. It was also observed that on the addition of SDS, the onset of the formation of polymer–surfactant complexes was insensitive to the degree of PEI modification, but the authors did not addressed the influence of the length of hydrophobic tails. Therefore, in this study, we synthesized four hydrophobically modified PEIs (Scheme 1) by partial alkylation of PEI with 1-bromobutane (PEI-C4), 1-bromohexane (PEI-C6), 1-bromooctane (PEI-C8), and 1-bromododecane (PEI-C12). The physicochemical properties of these decorated PEIs were then studied in aqueous solution in the absence and in the presence of SDS with the use of viscosity, surface tension, fluorescence, and small-angle X-ray scattering (SAXS) techniques. All data were explained with the application of a model that considers the interaction of the polymer and the surfactant by means of combined hydrophobic and electrostatic effects.

2. Experimental section

2.1. Materials and methods

SDS was supplied by Sigma-Aldrich with a purity of 99% and was used without further purification. Pyrene (Sigma-Aldrich, 99%) was recrystallized three times from methanol and dried before use. PEI with a nominal molecular weight of 25,000 g mol^{-1} was obtained as a pure polymer from Sigma-Aldrich. The primary/secondary/tertiary amine ratios for these polymers were 1:2:1, which corresponds to a branch every 3–3.5 nitrogens. 1-Bromobutane, 1-bromohexane, 1-bromooctane, and 1-bromododecane, all with purity of 99%, were purchased from Sigma-Aldrich and used as received. Deionized water, obtained through previous distillation followed by purification employing a Millipore Milli-Q system, was used in all measurements. This solvent was boiled and bubbled with nitrogen and kept in a nitrogen atmosphere to avoid the presence of carbon dioxide. Stock solutions of PEI and alkylated PEI were prepared in water under magnetic stirring for at least 12 h.

2.2. Synthesis and characterization of the alkylated PEIs

The following general methodology, adapted from Park et al. [31], was applied to prepare the alkylated PEIs: the 1-bromoalkane (12.0 mmol) was added drop by drop to a vigorously stirred solution of PEI (5.28 g; 120.0 mmol per monomer) in 65.0 mL of 2-methylpropan-2-ol containing an excess of potassium carbonate (20.73 g; 150.0 mmol) [31]. The reaction mixture was stirred at room temperature for 5 days. Subsequently, the reaction mixture was placed into a dialysis membrane (Spectra/Por® 6, MWCO = 3500 g mol^{-1}) and dialyzed for three days to remove unreacted 1-bromoalkane, 2-methylpropan-2-ol, and potassium carbonate. After, the resulting aqueous solution was lyophilized, and the product was characterized by ^{1}H NMR. The absence of the NMR signals corresponding to the protons of 1-bromoalkane and 2-methylpropan-2-ol in the product confirmed the purity of the product. ^{1}H NMR for PEI-C4 (400 MHz, $CDCl_3$): δ 0.89 (*t*, $\mathbf{H_3C}CH_2CH_2CH_2NH–$), δ 1.30 (*m*, $H_3C\mathbf{CH_2}CH_2$ $CH_2NH–$), δ 1.40 (*m*, $H_3CCH_2\mathbf{CH_2}CH_2NH–$), δ 2.39 (*m*, $H_3CCH_2CH_2\mathbf{CH_2}NH–$), δ 2.5–2.8 (*m*, $–NH(\mathbf{CH_2})_2NH–$), δ 4.00 (*bs*, –NH–); ^{1}H–NMR for PEI-C6 (400 MHz, $CDCl_3$): δ 0.83 (*t*, $\mathbf{H_3}$ $\mathbf{C}(CH_2)_3CH_2CH_2NH–$), δ 1.23 (*m*, $H_3C(\mathbf{CH_2})_3CH_2CH_2NH–$), δ 1.41 (*m*, $H_3C(CH_2)_3\mathbf{CH_2}CH_2NH–$), δ 2.35 (*m*, $H_3C(CH_2)_3CH_2\mathbf{CH_2}NH–$), δ 2.5–2.8 (*m*, $–NH(\mathbf{CH_2})_2NH–$), δ 4.00 (*bs*, –NH–); ^{1}H NMR for PEI-C8 (400 MHz, $CDCl_3$): δ 0.84 (*t*, $\mathbf{H_3C}(CH_2)_5CH_2CH_2NH–$), δ 1.23 (*m*, $H_3C(\mathbf{CH_2})_5CH_2CH_2NH–$), δ 1.43 (*m*, $H_3C(CH_2)_5\mathbf{CH_2}CH_2NH–$), δ 2.37 (*m*, $H_3C(CH_2)_5CH_2\mathbf{CH_2}NH–$), δ 2.5–2.8 (*m*, $–NH(\mathbf{CH_2})_2NH–$), δ 4.30 (*bs*, –NH–); ^{1}H NMR for PEI-C12 (400 MHz, $CDCl_3$): δ 0.85 (*t*, $\mathbf{H_3C}(CH_2)_9CH_2CH_2NH–$), δ 1.23 (*m*, $H_3C(\mathbf{CH_2})_9CH_2CH_2NH–$), δ 1.45 (*m*, $H_3C(CH_2)_9\mathbf{CH_2}CH_2NH–$), δ 2.38 (*m*, $H_3C(CH_2)_9CH_2\mathbf{CH_2}NH–$), δ 2.5–2.8 (*m*, $–NH(\mathbf{CH_2})_2NH–$), δ 4.17 (*bs*, –NH–).

2.3. pH measurements

The pH values were obtained in the following way: an aqueous solution of the PEI (2.0 mg mL^{-1}) was prepared and used to make a stock solution of SDS in a concentration of 4.0×10^{-2} mol L^{-1}. The PEI solution was kept in a thermostated cell at 25.0 ± 0.1 °C, and small amounts of aqueous stock solutions of SDS were added with a semi-automatic burette Metrohm Herisau (multi-burette type model E-485). After each addition of stock solution, the pH was measured by an immersed pH electrode (Beckman ϕ 71 pH meter with a combined glass electrode).

2.4. Viscosity measurements

Viscosity measurements were performed on a SCHOTT AVS 350 viscometer (capillary viscometer *Cannon–Fenske,* diameter 0.54 mm) equipped with an optical system for flow detection. The temperature was controlled by a water bath and maintained at 25.0 ± 0.1 °C. For the measurements, stock solutions of each polymer (10.0 mg mL^{-1}) were prepared in deionized water and used to prepare 10.0 mL of each more dilute solution.

2.5. Surface tension measurements

For the surface tension measurements, the du Nouy ring method was used and the experiments were carried out at 25.0 ± 0.1 °C on a Kruss K8 GMBH interfacial tensiometer equipped with a Pt–Ir-20 ring. The ring was rinsed with a hydrochloric acid solution (4.0 mol L^{-1}) and rinsed with deionized water several times before each measurement. All solutions were prepared in the same manner as described in the previous subsection. All polymer solutions prepared were left to rest for at least two hours before use.

2.6. Steady-state fluorescence

The steady-state fluorescence emission spectra of pyrene were recorded on a Hitachi F4500 spectrofluorimeter equipped with a thermostated cell holder set at 25.0 ± 0.1 °C, and the samples were continuously stirred in a quartz cell of 10 mm path length. The concentration of the probe was fixed at 1.0×10^{-6} mol L^{-1} to avoid the formation of pyrene microcrystals and extrinsic phenomena. Both slit width settings of excitation and emission monochromators were adjusted to 2.5 nm. The samples were excited at 336 nm, and the emission spectra were recorded from 360 to 500 nm. The following procedure was applied for all experiments performed. The fluorescence spectrum was recorded after the addition of each volume of surfactant solution. The I_1/I_3 ratio was considered as the ratio between the maximum peak emission intensities of the probe at 372.8 nm (I_1) and 384.0 nm (I_3).

2.7. SAXS experiments

All SAXS studies were carried out on the D11A-SAXS beamline of the Brazilian Synchrotron Light Laboratory (LNLS – Campinas – Brazil). The wavelength (λ) of the incoming beam was set at 0.1488 nm, and the samples were injected into a 1 mm thick sample [32]. The collimated beam crossed the samples through an evacuated flight tube ($P < 0.1$ mbar) and was scattered to a 2D CCD marCCD detector with an active area of 16 cm^2. The sample-to-detector distance was set at 1479.75 mm (silver behenate was utilized for the calibration of the sample-to-detector distance due to its well-known lamellar structure, $d = 58.48$ Å). The q range covered at this distance was 0.10–2.3 nm^{-1}. 2D images were found to be isotropic and were corrected by taking into account the detector dark noise and normalized by the sample transmission. These images of the samples were subtracted from the corrected and normalized 2D image of the solvent, and the resulting images were then azimuthally integrated, considering the 360° scan to generate the final I as a function of q profiles. This procedure was performed with the use of the FIT2D software developed by Hammersley [33].

3. Results and discussion

3.1. Synthesis and characterization

The modified PEIs, PEI-C4, PEI-C6, PEI-C8, and PEI-C12, were prepared by an adapted method based on that described by Daewon et al. [31], by means of the alkylation of the PEI amino groups with the corresponding bromo-alkanes. The modified polymers were characterized by ^{1}H NMR. Fig. 1A shows the ^{1}H NMR spectrum for PEI, exhibiting the $-CH_2CH_2N-$ group at δ 2.1–3.0 ppm. The signal at δ 2.0 was attributed to the protons of amino groups and disappears on the addition of a drop of deuterated water to the NMR tube. The ^{1}H NMR spectrum for PEI-C12 in Fig. 1B shows three signals between δ 0.80 and 2.0 ppm (absent in the spectrum of PEI), which related to the dodecyl groups linked to the amino groups, specifically at δ 0.85 (a, $\mathbf{H_3C}(CH_2)_9CH_2CH_2NH-$), δ 1.23 (b, $H_3C(\mathbf{CH_2})_9CH_2CH_2NH-$), and δ 1.45 (c, $H_3C(CH_2)_9\mathbf{CH_2}CH_2NH-$). In addition, a signal at δ 2.38 (d) was attributed to the methylene protons of the alkyl chain directly bonded to the nitrogen, being immediately adjacent to the region of the PEI ethylene groups $-NH(\mathbf{CH_2})_2NH-$ (e, δ 2.5–2.8). This was confirmed through the analysis of a homonuclear correlation spectroscopy ($^1H \times {}^1H$ COSY) spectrum, which revealed that protons at δ 2.38 and δ 1.45 are spin–spin coupled to each other. In addition, our observations are very similar to the description of a derivatized PEI by Cheng et al. [16]. With the alkylation, the signal corresponding to the presence of PEI NH_2 groups at δ 2.0 disappears, with the appearance of a new signal at δ 4.17, due to the presence of the alkylated NH groups. This was confirmed by the fact that the addition of deuterated water to the solution of the alkylated PEI led to the complete disappearance of the signal at δ 4.17. Thus, although in principle the alkylation can also occur at the secondary and tertiary amino groups, these data show that the primary amino groups were preferentially alkylated.

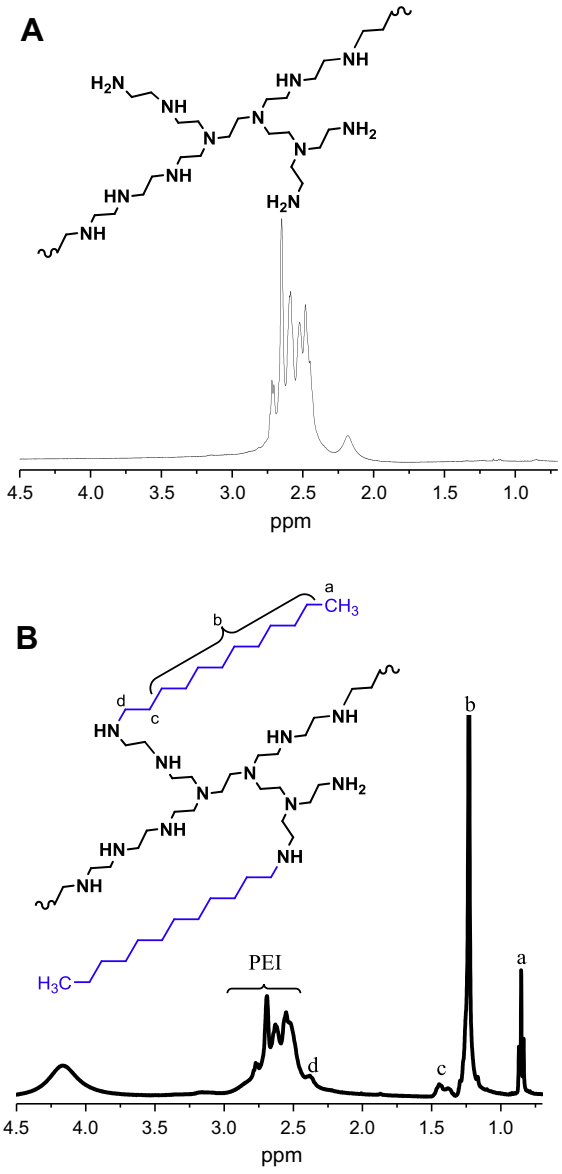


Fig. 1. ^{1}H NMR spectra of PEI (A) and PEI-C12 (B) (a, $\mathbf{H_3C}(CH_2)_9CH_2CH_2NH-$; b, $H_3C(\mathbf{CH_2})_9CH_2CH_2NH-$; c, $H_3C(CH_2)_9\mathbf{CH_2}CH_2NH-$; d, $H_3C(CH_2)_9CH_2\mathbf{CH_2}NH-$) in $CDCl_3$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The degree of modification in each derivatized PEI could be estimated from the integration of the peak areas corresponding to the $-CH_2CH_2N-$ and $-CH_3$ groups. The substitution degrees found for PEI-C4, PEI-C6, PEI-C8, and PEI-C12 were 9.2%, 9.2%, 9.1%, and 9.8%, respectively.

3.2. Surface tension and fluorescence measurements

Fig. 2 shows the surface tension values for PEI, PEI-C4, PEI-C6, PEI-C8, and PEI-C12 as a function of polymer concentration. While PEI showed no surface activity, the decorated PEIs exhibit a very different behavior, since these data show that the surface tension decreases with an increase in the polymer concentration. This behavior is similar to those observed with classical surfactants in that surface tension shows a decrease with an increase in *c*(surfactant) up to a constant surface tension value. A similar behavior was observed by Griffiths et al. [30] in a study on PEI synthetically modified with 1% and 10% of 1,2-epoxydodecane.

The surface tension studies motivated a similar investigation using the fluorescence technique and pyrene as a probe. Pyrene has been largely used as a fluorescence probe in the study of micelles [34,35] and lipophilic environments [36,37], in solvation studies [38–40] and in the investigation of aqueous mixtures of polyelectrolytes and surfactants [41–43]. Its use as a probe results from the fact that the intensities of the various fluorescence emission vibronic bands of the compound have been shown to be strongly dependent on the medium microenvironment [44,45]. More specifically, an enhancement in the intensity of the first fluorescence emission band (I_1) is observed in the presence of polar solvents, while only a slight effect is observed on the third band (I_3) [44,45]. Therefore, the I_1/I_3 ratio represents the basis for the pyrene scale of solvent polarities [46], a value of 1.8 being observed for water and 0.6 for hexane. Considering, for instance that a process of micellization occurs in water on the addition of a surfactant above its *cmc* value, pyrene, due to its lipophilic nature, migrates from the aqueous environment to the nonpolar micellar cores with the formation of micelles. The change in the polarity that occurs in the microenvironment of the probe is expressed by the lowering of the I_1/I_3 ratio, making it possible to follow the aggregation phenomenon.

Fig. 3 shows the I_1/I_3 ratio for pyrene fluorescence emission as a function of the concentration of PEI and modified PEIs. Firstly, it is important to remark that for the commercial PEI, the I_1/I_3 ratio exhibits a very small change with an increase in the PEI concentration, remaining in the range of 1.68–1.80, which is consistent with the reported value in water [44], suggesting that PEI is not able to offer hydrophobic domains. Interestingly, for modified PEIs, the I_1/I_3 values decrease with an increase in the polymer concentration from 1.80 in pure water to a minimum I_1/I_3 value, which is dependent on the alkyl group bonded to the PEI. At low polymer concentrations, more significant decreases were observed for PEI-C8 and PEI-C12 compared to PEI-C4 and PEI-C6. The minimum I_1/I_3 values observed in each system were 1.14, 1.03, 0.95, and 0.88 for PEI-C4, PEI-C6, PEI-C8, and PEI-C12, respectively, indicating that pyrene migrates to an increasingly hydrophobic domain with increases in the alkyl chain of the modified PEI. Thus, pyrene fluorescence shows that there are significant differences between the modified and unmodified polymer. In addition, a comparison between the surface tension and pyrene fluorescence data reveals that the discontinuity in the surface tension data coincides with the region in which the decrease in I_1/I_3 values occurs (~3.0 mg mL^{-1}), which suggests

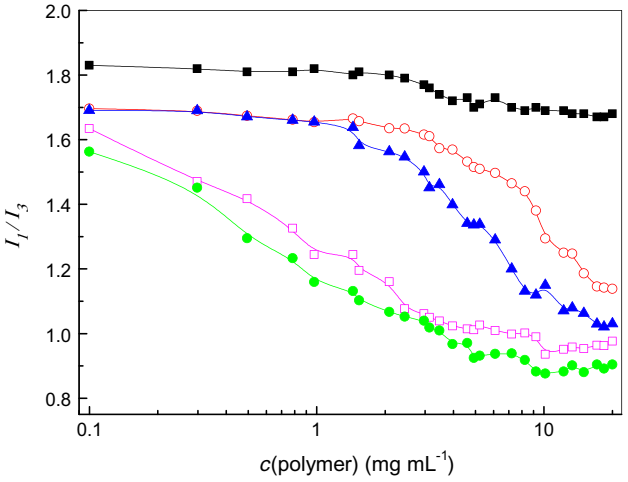


Fig. 3. I_1/I_3 ratio for pyrene fluorescence emission with increasing concentrations of PEI (■), PEI-C4 (○), PEI-C6 (▲), PEI-C8 (□), and PEI-C12 (●). The concentration of pyrene was 1.0×10^{-6} mol L^{-1}, with excitation at 336 nm, and the slits adjusted to 2.5 nm at 25.0 ± 0.1 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

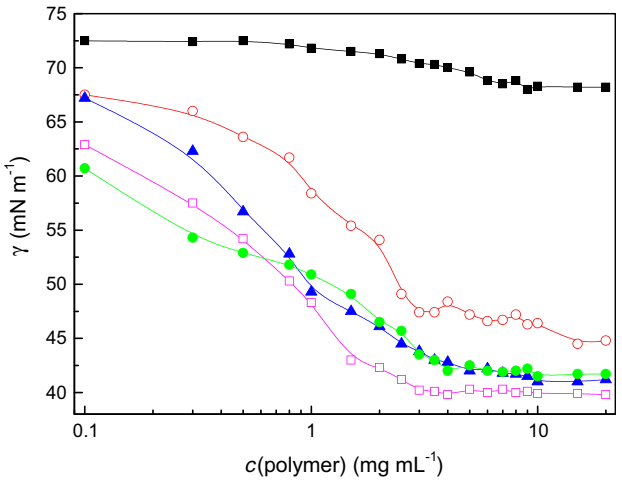


Fig. 2. Surface tension for increasing concentrations of PEI (■), PEI-C4 (○), PEI-C6 (▲), PEI-C8 (□), and PEI-C12 (●) at 25.0 ± 0.1 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

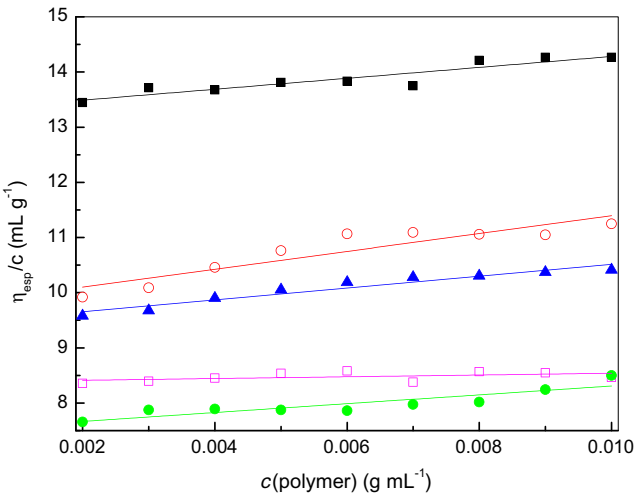


Fig. 4. Influence of the polymer concentration on the specific viscosity at 25.0 ± 0.1 °C for the aqueous solutions of PEI (■), PEI-C4 (○), PEI-C6 (▲), PEI-C8 (□), and PEI-C12 (●). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the same physicochemical process, that is, the formation of the hydrophobic aggregates in aqueous solution.

3.3. Viscosity data

Fig. 4 shows the changes in the specific viscosity for solutions of PEI and modified PEIs as a function of the polymer concentration. It is clearly observed from analysis of the experimental data that the specific viscosities decrease with an increase in the alkyl chain of the PEI. These data also allow the determination of the intrinsic viscosities for PEI and modified PEIs, and it being observed that their values decreased with an increase in the alkyl chain. The intrinsic viscosities (η for the polymers in solution were determined (Table 1). The η value for PEI is 13.30 mL g^{-1}, and for the modified PEIs, the values decreased in the following order: PEI-C4 > PEI-C6 > PEI-C8 > PEI-C12.

It is important to mention that water is an efficient solvent in which to dissolve PEI, since the polymer is present in a protonated form. As a result, the chain of the polymer is stretched, which increases its hydrodynamic volume. On the other hand, due to their higher hydrophobicity, increasing from PEI-C4 to PEI-C12, the water solubility of the alkylated polymers is diminished and their molecules are found in the coiled form, which is responsible for a decrease in the intrinsic viscosity. Consequently, the overlap concentrations (c^*) for the modified polymers (Table 1), calculated as the reciprocal of each η value, increase with the size of their appended *n*-alkylic chains, that is, in the following order: PEI < PEI-C4 < PEI-C6 < PEI-C8 < PEI-C12.

3.4. Influence of SDS on the pH of PEI and modified PEI aqueous solutions

Since the addition of SDS would be expected to change the pH of polyelectrolyte solutions [21,23], the pH value of the aqueous solutions of PEI and of the modified PEIs in a concentration of 2.0 mg mL^{-1} in the absence and in the presence of increasing amounts of SDS was monitored (Fig. 5). All samples are in aqueous solutions with pH close to 9.5 when only in the presence of PEI or alkylated PEIs, and this value can increase to around 11.5 when the SDS concentration is 20 mmol L^{-1}. The aqueous solutions of the PEIs are alkaline, since the amine groups present in the polymer act as a weak base (Eq. (1)). When SDS is added, there is an increase in the pH value of the solution, due to the role of the anionic surfactant in stabilizing the weak conjugate acid, through a specific binding of SDS with the positively charged sites on the polymeric chains, which displaces the acid-base equilibrium, forming more hydroxide

$$R_3N_{(aq)} + H_2O_{(l)} \rightleftharpoons R_3NH^+_{(aq)} + {}^-OH_{(aq)} \quad (1)$$

3.5. Fluorescence measurements of PEI and modified PEI solutions in the absence and in the presence of SDS

Fig. 6 reports the use of pyrene as a probe to investigate the behavior of PEI and the modified PEIs as a function of SDS concentration, with the polymer concentration fixed at 2.0 mg mL^{-1}. It is

Table 1
Values for intrinsic viscosity (η and overlap concentration (c^*) for the modified PEIs at 25.0 ± 0.1 °C (for details on their determination, see the text).

Polymer	η (mL g^{-1})	c^* (mg mL^{-1})
PEI	13.30	75.20
PEI-C4	9.78	102.20
PEI-C6	9.44	105.95
PEI-C8	8.20	121.95
PEI-C12	7.50	133.30

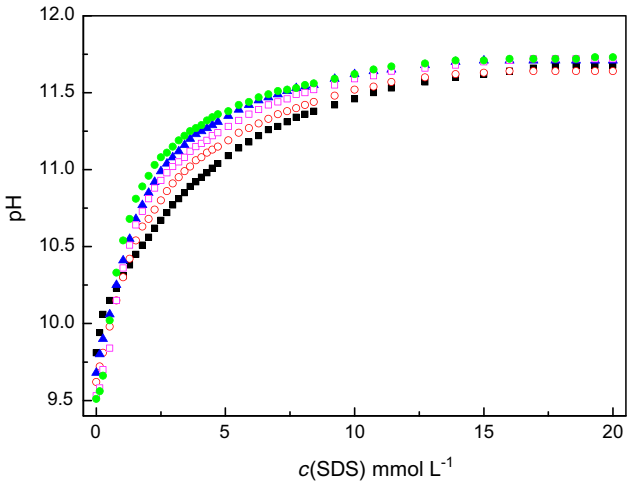


Fig. 5. Influence of the addition of SDS on the pH of aqueous solutions of PEI (■), PEI-C4 (○), PEI-C6 (▲), PEI-C8 (□), and PEI-C12 (●) (c(polymer) = 2.0 mg mL^{-1}) at 25.0 ± 0.1 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

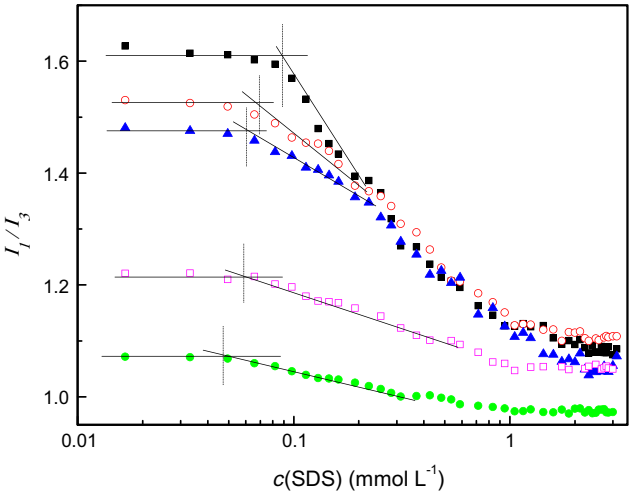


Fig. 6. Influence of I_1/I_3 ratio on pyrene fluorescence emission of aqueous solutions of PEI (■), PEI-C4 (○), PEI-C6 (▲), PEI-C8 (□), and PEI-C12 (●) (c(polymer) = 2.0 mg mL^{-1}) as a function of SDS concentration. The pyrene concentration was 1.0 × 10^{-6} mol L^{-1}, with excitation at 336 nm, and the slits adjusted to 2.5 nm at 25.0 ± 0.1 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

observed that for each polymer, at low concentrations, the I_1/I_3 ratio for PEI is ~1.60, but a gradual decrease occurs with an increase in the alkyl chain to a value of ~1.07 for PEI-C12. The same experiments were performed for the PEIs in a concentration of 0.1 mg mL^{-1}, which is below the *cac* of the PEIs, and the same trends could be verified. A similar behavior was observed by Magny et al. [47] in a study on mixed micellar systems comprising dodecyltrimethylammonium chloride and a series of hydrophobically modified poly(sodium acrylate). This behavior is due to the fact that the concentration of hydrophobic domains in the modified polymers increases with an increase in their alkyl chains. These hydrophobic domains also help to initiate the polymer–surfactant association. Thus, the slight decrease in the I_1/I_3 ratio observed at lower concentrations of SDS indicates the onset of the polymer–surfactant interaction. This discontinuity in the I_1/I_3 ratio is called the critical aggregate concentration (*cac*), and it starts at a lower concentration than the *cmc* of the pure SDS solution (~8.0 mmol L^{-1}) [48]. Table 2 shows the *cac* values for PEI and modified PEIs, it being observed

Table 2
Values for *cac* of PEI and modified PEIs with SDS, obtained by means of the use of a pyrene fluorescence probe.

Polymer	*cac* (mmol L^{-1})
PEI	0.090
PEI-C4	0.070
PEI-C6	0.060
PEI-C8	0.055
PEI-C12	0.045

that the values gradually decrease with an increase in the alkylic chain of the polymer. For instance, the *cac* for the PEI/SDS system is ~0.09 mmol L^{-1}, decreasing to ~0.045 mmol L^{-1} for the PEI-C12/SDS system. Therefore, the modified polymers, having amphiphilic properties, changed the onset of the polymer–surfactant association toward lower concentrations of surfactant. This finding is similar to results obtained by Magny et al. [47], who also observed that the longer the alkylic chain the lower the *cac* value.

3.6. SAXS measurements of modified PEI solutions in the absence and in the presence of SDS

PEI and alkylated PEIs were investigated with the use of the SAXS technique, maintaining a constant polymer concentration of 2.0 mg mL^{-1}. These studies were performed in order to evaluate possible morphological evolutions in the modified polymers in comparison with the unmodified polymer.

Fig. 7 reports the behavior of PEI (A) and PEI-C12 (B) in the absence of SDS and with three different SDS concentrations (5.0, 10.0, and 20.0 mmol L^{-1}). The displacement of the 'bump' toward the high q region as the c(surfactant) increases provides a qualitative indication that the complexes are smaller at higher concentrations. Similar behavior was observed recently by Felippe et al. [29] in a study on the formation of supramolecular complexes composed of PEI, sodium cholate, and SDS. These data suggest that the size of the aggregates may be influenced by an increase in the counter

Table 3
Values of the radius of gyration for PEI and modified PEIs.

c(SDS) (mmol L^{-1})	R_g (nm)				
	PEI	PEI-C4	PEI-C6	PEI-C8	PEI-C12
0.0	16.30	14.30	15.10	14.70	14.50
5.0	14.70	14.00	14.30	14.30	14.10
10.0	14.50	13.80	14.20	13.90	13.70
20.0	13.90	13.20	13.50	13.70	13.30

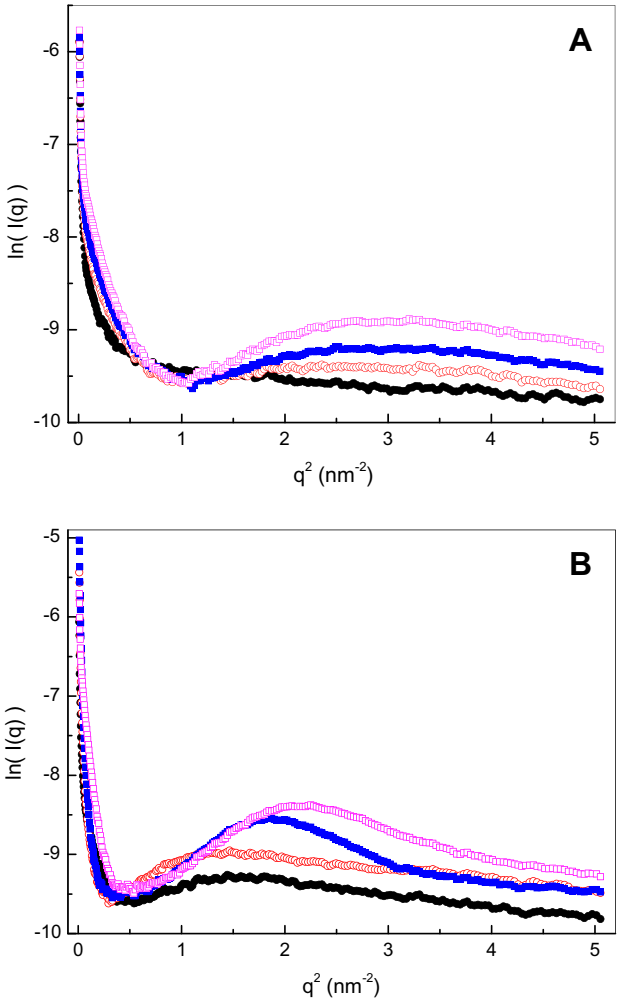


Fig. 7. SAXS patterns measured for (A) PEI and (B) PEI-C12 in the absence (●) and in the presence of SDS at 5.0 (○), 10.0 (■), and 20.0 (□) mmol L^{-1}. The concentration of each polymer was 2.0 mg mL^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

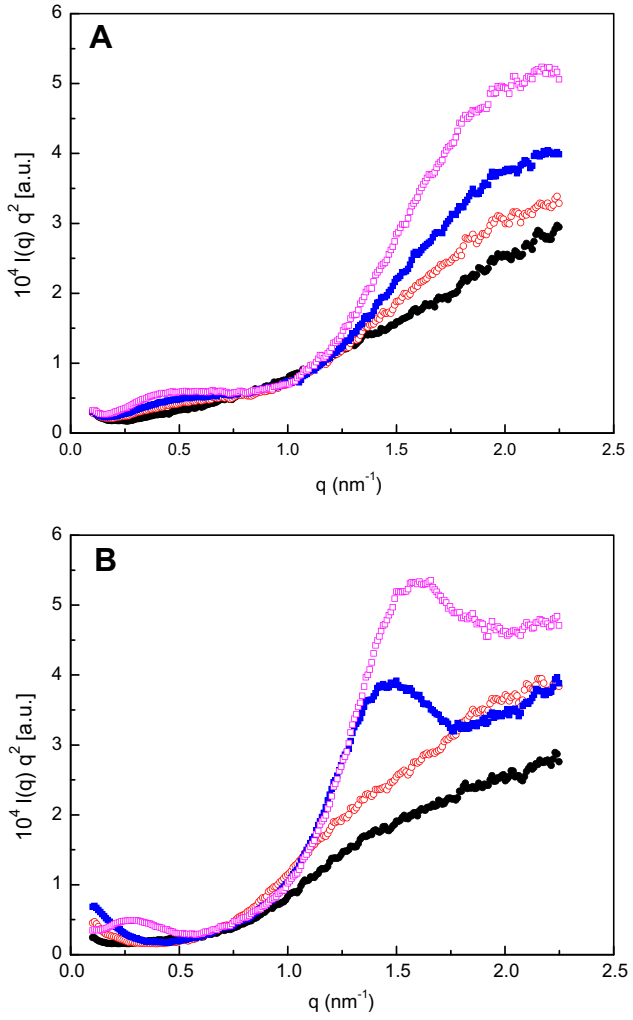
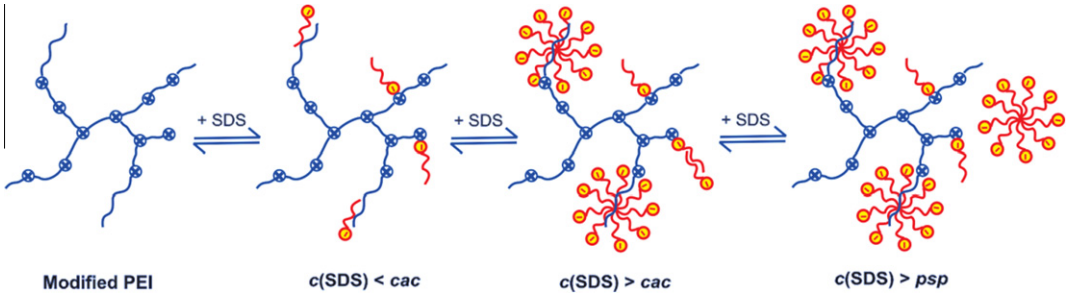


Fig. 8. Kratky plots of (A) PEI (2.0 mg mL^{-1}) (●) in aqueous solutions in the presence of 5.0 mmol L^{-1} (○), 10.0 mmol L^{-1} (■), and 20.0 mmol L^{-1} (□) of SDS; (B) PEI-C12 (2.0 mg mL^{-1}) (●) and PEI-C12 with 5.0 mmol L^{-1} (○), 10.0 mmol L^{-1} (■), and 20.0 mmol L^{-1} (□) of SDS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Scheme 2. Schematic representation of the steps involved the association of the modified polymers with SDS.

ions in solution promoted by an increase in the concentration of the surfactant. This would promote repulsion between the aggregates, which consequently would influence their size. In addition, the increase in the concentration of counter ions could shield electrostatic repulsion between ionic side chains of the polymers, resulting in a more compact polymer conformation.

The Guinier's approximation [49] was used in the determination of the radius of gyration of the polymers under each condition, from the plots of $\ln(I(q))$ as a function of q^2, by linear fitting these data at the limit $q \to 0$. The results are presented in Table 3, and for PEI, there is a clear reduction in the radius of gyration (R_g) of the polymer, from 16.30 in the absence of surfactant to 13.90 nm in the presence of 20 mmol L^{-1} of SDS. The decrease in the value of R_g is also observed for all modified polymers, with the results indicating a general decrease in the values with the increasing alkyl substituent in PEI. These data suggest that the modified polymer chains are coiled in solution in comparison with the unmodified PEI (see discussion above). These values of R_g are different of that reported by Pfau et al. [50], who showed using light scattering experiments that a PEI of 37 kDa, at pH 4.0 and adsorbed to mica surface, had a diameter of 6 nm. The high R_g values calculated for the PEIs are due to the high pH values used in our study, varying from 9.5 in the absence to 11.5 in the presence of SDS, which leads to polymers with a very elongated structure. A similar effect was reported by Griffiths et al. [30] who used small-angle neutron scattering (SANS) at pH 10. In other study, using the static light scattering technique, Bastardo et al. [26] obtained for PEI of 70 kDa in D_2O solutions (pD 10.1) an R_g value of 55 nm. This value is proportionally similar to our results, if the molecular weight of the polymers used in the measurements is compared.

The Kratky plots $I(q)\,q^2$ versus q of the SAXS intensity functions for samples with branched PEI and PEI-C12 with 5.0, 10.0, and 20.0 mmol L^{-1} of SDS are shown in Fig. 8, where different sample properties can be clearly observed. The solutions of pure polymers and those in the presence of 5.0 mmol L^{-1} of SDS exhibit roughly the same behavior; the Kratky curves for these systems show a monotonic increase, indicating a flexible random-coil conformation [51]. When the concentration of SDS is increased above the *cmc* (>8 mmol L^{-1}), the solution of branched PEI continues to exhibit the same behavior of random-coil conformation (only an increase in the overall scattering intensity is observed). However, a peak appears in the solutions of PEI-C12 when the *c* (SDS) is greater than the *cmc*. This behavior can be interpreted by analogy to protein solutions: folded globular proteins typically yield a prominent peak, whereas unfolded proteins show a continuous increase in $I(q)\,q^2$ with q [52]. Thus, the peak can indicate the formation of compact aggregates when the PEI has hydrophobic branches, in the presence of a high concentration of surfactant. In these systems, the curves show not only the peak, but also the increase in $I(q)\,q^2$ with q, indicating the presence of multi-domains, displaying a mixture of the characteristic features of free SDS micelles and both aggregated and random-coil polymer chains [53,54].

The main features related to the system studied here, involving the interaction of the alkylated PEIs and SDS in aqueous solution, are summarized in Scheme 2. Firstly, the addition of SDS to a solution of the modified polymer leads to the interaction by means of hydrophobic and electrostatic effects. With an increase in the concentration of SDS to a value greater than the *cac*, aggregates of SDS can interact with the polymer through combined electrostatic and hydrophobic effects, the extent of this interaction being dependent on the size of the alkylic chain. After the saturation of the polymer surface with the aggregates of SDS, that is, above the polymer saturation point (*psp*), the increase in the concentration of SDS leads to the appearance of surfactant-free micelles in equilibrium with the SDS-modified PEI aggregates.

4. Conclusions

The systematic study performed here, by means of the synthetic modification of PEI with alkyl groups, resulted in polymers exhibiting a pronounced surface activity, similar to values reported for typical surfactants [30,55], leading to the formation of hydrophobic domains [30]. These data obtained are consistent with reports published in the recent literature [30], and in addition, our study shows clearly that the hydrophobicity of PEI can be tuned by increasing the length of the alkyl groups. The trends observed here are of interest for application in the development of systems tailored to be soluble in aqueous environments but also able to interact with lipophilic or amphiphilic species.

This was verified through observing the interaction of hydrophobically modified polymers with SDS. The association of PEI and modified PEI with SDS showed that an increase in the surfactant concentration contributes to a systematic reduction in the R_g value, suggesting that the modified polymers are coiled in solution in comparison with the unmodified PEI. It is interesting also to note for PEI-C12 in aqueous solution, in the presence of a high concentration of SDS, the formation of compact aggregates, displaying the presence of multi-domains between aggregated and random-coil polymer chains. Griffiths et al. [30] showed that an increase in the degree of modification in PEI led to the formation of polymers with pronounced surface activity, with no changes in the onset of the formation of complexes involving the polymer and surfactants upon addition of SDS. Our study showed that considering the same degree of PEI modification, but increasing the length of the alkylic substituent chain, an increase in the surface activity was obtained, with the onset of the formation of polymer–surfactant aggregates occurring at minor SDS concentrations.

The different techniques employed were found to be ideal for gaining insight into these systems, and a model for the interaction of the PEIs with SDS in water could be proposed, based on the combined hydrophobic and electrostatic effects. This is of great importance in terms of shedding light on our studies involving the design of systems able to interact with DNA, based on the combination of

modified PEIs with SDS and also in the design of catalyst supports. Therefore, the strategy of using hydrophobically modified PEIs with anionic surfactants could potentially be applied in the development of systems aiming at the encapsulation of dyes [56], gene delivery therapy [3,4], drug delivery [16], and cosmetic products [14].

Acknowledgments

We acknowledge the Brazilian Synchrotron Light Laboratory (LNLS) for the beamtime under proposal D11A-SAXS1-9363 and the Brazilian government agency Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

References

[1] C. Note, J. Koetz, L. Wattebled, A. Laschewsky, J. Colloid Interface Sci. 308 (2007) 162.
[2] J.C. Roberts, Paper Chemistry, Blackie Academic & Professional, Chapman & Hall, London, 1996.
[3] M.L. Forrest, J.T. Koerber, D.W. Pack, Bioconjugate Chem. 14 (2003) 934.
[4] M. Thomas, A.M. Klibanov, Appl. Microbiol. Biotechnol. 62 (2003) 27.
[5] D.R. Coleman, G.P. Royer, J. Org. Chem. 45 (1980) 2268.
[6] A. Haimov, H. Cohen, R. Neumann, J. Am. Chem. Soc. 126 (2004) 11762.
[7] Z. Shen, Y. Chen, H. Frey, S.-E. Stiriba, Macromolecules 39 (2006) 2092.
[8] A.M. Signori, K.d.O. Santos, R. Eising, B.L. Albuquerque, F.C. Giacomelli, J.B. Domingos, Langmuir 26 (2010) 17772.
[9] R. Deng, Y. Yue, F. Jin, Y. Chen, H.-F. Kung, M.C.M. Lin, C. Wu, J. Control. Release 140 (2009) 40.
[10] A. Zintchenko, A. Philipp, A. Dehshahri, E. Wagner, Bioconjugate Chem. 19 (2008) 1448.
[11] F.M. Winnik, S.T.A. Regismond, Colloid Surface A 118 (1996) 1.
[12] U. Gupta, H.B. Agashe, A. Asthana, N.K. Jain, Biomacromolecules 7 (2006) 649.
[13] N. Gunnar, H. Walter, Macromol. Chem. Phys. 199 (1998) 1637.
[14] M. Gamez-Garcia, R.R. Meyer, Delivery System Handbook for Personal Care and Cosmetic Products, William Andrew Publishing, Norwich, NY, 2005 (p. 501).
[15] L. Zhang, C.–H. Hu, S.–X. Cheng, R.–X. Zhuo, Colloid Surfaces B. 76 (2010) 427.
[16] W.P. Cheng, A.I. Gray, L. Tetley, T.L.B. Hang, A.G. Schätzlein, I.F. Uchegbu, Biomacromolecules 7 (2006) 1509.
[17] S.-H. Park, S. Wei, B. Mizaikoff, A.E. Taylor, C. Favero, C.-H. Huang, Environ. Sci. Technol. 43 (2009) 1360.
[18] J. Koetz, S. Kosmella, Polyelectrolytes and Nanoparticles, Springer, Berlin, 2007.
[19] B. Bolto, J. Gregory, Water Res. 41 (2007) 2301.
[20] S.M. Bystryak, M.A. Winnik, J. Siddiqui, Langmuir 15 (1999) 3748.
[21] M.A. Winnik, S.M. Bystryak, C. Chassenieux, V. Strashko, P.M. Macdonald, J. Siddiqui, Langmuir 16 (2000) 4495.
[22] R. Mészáros, L. Thompson, M. Bos, I. Varga, T. Gilányi, Langmuir 19 (2003) 609.
[23] R. Mészáros, J. Colloid Interface Sci. 338 (2009) 444.
[24] A. Sakar-Deliormanh, J. Disper. Sci. Technol. 31 (2009) 23.
[25] A. Mezei, Á. Ábrahám, K. Pojják, R. Mészáros, Langmuir 25 (2009) 7304.
[26] L.A. Bastardo, V.M. Garamus, M. Bergström, P.M. Claesson, J. Phys. Chem. B 109 (2004) 167.
[27] L.A. Bastardo, R. Mészáros, I. Varga, T. Gilányi, P.M. Cleasson, J. Phys. Chem. B 109 (2005) 16196.
[28] A. Dedinaite, R. Mészaros, P.M. Claesson, J. Phys. Chem. B 108 (2004) 11645.
[29] A.C. Felippe, I.C. Bellettini, R. Eising, E. Minatti, F.C. Giacomelli, J. Braz. Chem. Soc. 22 (2011) 1539.
[30] P.C. Griffiths, A. Paul, I.A. Fallis, C. Wellappili, D.M. Murphy, R. Jenkins, S.J. Waters, R. Nilmini, R.K. Heenan, S.M. King, J. Colloid Interface Sci. 314 (2007) 460.
[31] D. Park, J. Wang, A.M. Klibanov, Biotechnol. Prog. 22 (2006) 584.
[32] L.P. Cavalcanti, I.L. Torriani, T.S. Plivelic, C.L.P. Oliveira, G. Kellermann, R. Neuenschwander, Rev. Sci. Instrum. 75 (2004) 4541.
[33] A.P. Hammersley, Scientific Software FIT 2D (2009).
[34] K. Kalyanasundaram, M. Graetzel, J.K. Thomas, J. Am. Chem. Soc. 97 (1975) 3915.
[35] R. Zana, Surfactant Solutions, Marcel Dekker, New York, 1986.
[36] V. Glushko, C. Karp, M. Sonenberg, Biophys. J. 16 (1976) A48.
[37] V. Glushko, M.S.R. Thaler, C.D. Karp, Arch. Biochem. Biophys. 210 (1981) 33.
[38] W.E. Acree, S.A. Tucker, D.C. Wilkins, J. Phys. Chem. 97 (1993) 11199.
[39] Y. Kusumoto, Y. Takeshita, J. Kurawaki, I. Satake, Chem. Lett. 26 (1997) 349.
[40] M.A.doR. Silva, D.C. da Silva, V.G. Machado, E. Longhinotti, V.L.A. Frescura, J. Phys. Chem. A 106 (2002) 8820.
[41] K. Kogej, J. Skerjanc, Langmuir 15 (1999) 4251.
[42] X. Wang, Y. Li, J. Wang, Y. Wang, J. Ye, H. Yan, J. Zhang, R.K. Thomas, J. Phys. Chem. B 109 (2005) 12850.
[43] F. Petit-Agnely, I. Iliopoulos, R. Zana, Langmuir 16 (2000) 9921.
[44] K. Kalyanasundaram, J.K. Thomas, J. Am. Chem. Soc. 99 (1977) 2039.
[45] A. Nakajima, Bull. Chem. Soc. Jpn. 44 (1971) 3272.
[46] D.C. Dong, M.A. Winnik, Photochem. Photobiol. 35 (1982) 17.
[47] B. Magny, I. Iliopoulos, R. Zana, R. Audebert, Langmuir 10 (1994) 3180.
[48] D. Zanette, A.C. Felippe, B. Schweitzer, A. Dal Bo, A. Lopes, Colloid Surface A 279 (2006) 87.
[49] N. Stribeck, X-Ray Scattering of Soft Matter, Springer, 2007.
[50] A. Pfau, W. Schrepp, D. Horn, Langmuir 15 (1999) 3219.
[51] Y. Hagihara, M. Hoshino, D. Hamada, M. Kataoka, Y. Goto, Fold. Des. 3 (1998) 195.
[52] V.N. Uversky, D.J. Segel, S. Doniach, A.L. Fink, Proc. Natl. Acad. Sci. USA 95 (1998) 5480.
[53] V.M. Garamus, T.V. Maksimova, H. Kautz, E. Barriau, H. Frey, U. Schlotterbeck, S. Mecking, W. Richtering, Macromolecules 37 (2004) 8394.
[54] H.D.T. Mertens, D.I. Svergun, J. Struct. Biol. 172 (2010) 128.
[55] S. Lukashenko, A. Yurina, T. Pashirova, D. Kudryavtsev, E. Kosacheva, L. Kudryavtseva, A. Konovalov, Colloid J. 70 (2008) 317.
[56] H. Liu, Y. Chen, D. Zhu, Z. Shen, S.E. Stiriba, React. Funct. Polym. 67 (2007) 383.